

INFANT FORMULA CONTAINING NUCLEOTIDES

FIELD OF THE INVENTION

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This application claims priority from co-pending provisional application serial number 60/417,458, filed on October 10, 2002, the entire disclosure of which is hereby incorporated by reference.

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The present invention is directed to infant formula compositions which contain nucleotides. More specifically, the present invention relates to preterm or low birth weight infant formula containing nucleotides at levels suitable for feeding preterm infants.

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DESCRIPTION OF THE PRIOR ART

Preterm infants have increased nutrient demands in order to maintain the rapid early life development of body tissue. In pediatric care facilities, the survival of low birth weight infants (LBW > 1000g) and in particular, very low birth weight infants (VLBW < 1000g) has increased. However, the endogenous nutrient storage capabilities of these infants is severely limited.

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The premature infant is notably different from the term infant. The initial immaturity and subsequent rapid growth rate of the premature infant necessitates a higher nutrient intake than the term infant. During the past several years, nutritionists have made substantial progress regarding the establishment of the nutrient needs of premature infants and have adjusted the composition of preterm infant formulas accordingly. The composition of preterm infant formula is based primarily on the composition of human breast milk as well as on intra-uterine accretion. Preterm infant formulas have thus been modified to contain appropriately increased amounts of nutrients, such as protein and fat, as well as minerals, such as calcium, phosphorus, copper and zinc.

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Means for providing adequate nutrition to premature infants has long been desired. Efforts have been made to enrich standard infant formula in such a manner to enrich its nutrition capabilities particularly in the feeding of premature infants.

5 Nucleotides are found in all living cells. Mature human breast milk contains thirteen different nucleotides. The five nucleotides which appear in the highest concentration are cytidine monophosphate (CMP), guanosine monophosphate (GMP), adenosine monophosphate (AMP), uridine monophosphate (UMP), and inosine monophosphate (IMP). Human milk further comprises nucleosides, nucleotide polymers and nucleotide adducts capable of contributing to the Total
10 Nucleotide Pool (TNP). However, in the preterm infant, de novo synthesis and the salvage method for nucleotide formation may be inadequate.

Infant formulas for term infants have been fortified with nucleotides to simulate mother's milk and to treat certain specific conditions.

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For example, US Patent No. 5,492,899 discloses an infant formula containing CMP, UMP, AMP and GMP to provide a dietary formula that enhances the immune system and alleviates diarrhea.

20 US Patent No. 4,544,559 discloses a nucleotide enriched humanized milk for infant nourishment which contains AMP, CMP, GMP, IMP and UMP at specific levels.

US Patent Nos. 4,994,442 and 5,086,500 disclose the addition of nucleosides and nucleotides to infant formulas having enhanced physiological properties and
25 which closely resemble mother's milk.

However, the addition of nucleotides to infant formula to resemble the milk of mother's of preterm infants has not been disclosed. It is postulated that nucleotides obtained from the diet may be essential to the preterm infant playing an important
30 role in cell growth, energy transfer, and promotion of gut maturation and development. Nucleotides may also provide benefits relating to biological effects, including increasing beneficial gut microflora, improving lipid metabolism and enhancing immunity.

SUMMARY OF THE INVENTION

The present invention is directed to an infant formula composition comprising a total nucleotide concentration similar to that of the breast milk of mothers of
5 preterm infants. The present infant formula compositions comprise 3.2 mg/L to 15.4 mg/L of CMP; 1.8 mg/L to 11.0 mg/L of UMP; 1.8 mg/L to 8.0 mg/L of GMP; 0.1 mg/L to 2.2 mg/L of IMP; and 2.5 mg/L to 13.2 mg/L of AMP.

DETAILED DESCRIPTION OF THE INVENTION

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The present infant formula compositions have a total nucleotide concentration of 9.3 mg/L to 49.8 mg/L, preferably 12.4 mg/L to 37.4 mg/L and most preferably 15.5 mg/L to 24.9 mg/L.

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The present infant formula compositions comprise the following nucleotides in the stated amounts:

- 20 a) CMP: 3.2 mg/L to 15.4 mg/L, preferably 4.2 mg/L to 11.6 mg/L; and most preferably 5.3 mg/L to 7.7 mg/L;
- b) UMP: 1.8 mg/L to 11.0 mg/L, preferably 2.4 mg/L to 8.3 mg/L, and most preferably 3.0 mg/L to 5.5 mg/L;
- c) GMP: 1.8 mg/L to 8.0 mg/L, preferably 2.4 mg/L to 6.0 mg/L, and most preferably 3.0 mg/L to 4.0 mg/L;
- 25 d) IMP: 0.1 mg/L to 2.2 mg/L, preferably 0.1 mg/L to 1.7 mg/L, and most preferably 0.1 mg/L to 1.1 mg/L; and
- e) AMP: 2.5 mg/L to 13.2 mg/L, preferably 3.3 mg/L to 9.9 mg/L, and most preferably 4.1 mg/L to 6.6 mg/L.

The present infant formulas may be additionally formulated with other
30 nutritionally beneficial ingredients recognized in the art, such as polyunsaturated fatty acids, (e.g. arachidonic acid and docosahexaenoic acid). The present formulas may also contain all vitamins and minerals essential in the diet of the preterm infant. These vitamins and minerals should be present in nutritionally significant amounts.

Examples of vitamins and minerals which may be added to the present infant formula compositions include vitamin A, vitamin B complex, vitamin C, vitamin D, vitamin E, vitamin K, calcium, magnesium, sodium, potassium, phosphorous, copper, zinc, chloride, iodine, selenium, iron, niacin, folic acid, pantothenic acid, biotin, choline, inositol and manganese.

The infant formula compositions of the present invention may further comprise macronutrients beneficial to the preterm infant such as carotenoids, taurine, coconut oil, sunflower oil, soy oil, native or synthetic fats, palmitic acid, medium chain triglycerides, lactose, maltodextrin, glucose, bovine oligosaccharides or fractions thereof, skim milk, milk whey, soy protein, rice protein, protein hydrolysates and lactoferrin.

The present infant formulas, which are particularly suited for feeding the preterm or low birth weight infant, contain nucleotides at levels found in breast milk of mothers of preterm infants. These levels are quite different than the levels of nucleotides found in the breast milk of mothers of term infants.

The present infant formulas may be in the form of a liquid, either as a ready to feed liquid or as a concentrated liquid requiring dilution with water prior to feeding, or in a powder form requiring reconstitution with water prior to use.

The present infant formula may be prepared by blending appropriate quantities of whey protein concentrate with skimmed milk, lactose, vegetable oils and fat soluble vitamins in deionized water. Preferably, these materials are blended together in quantities sufficient to provide a final concentration of approximately 240 grams/liter. Mineral salts may then be added to the mixture prior to a high temperature/short time pasteurization step. Appropriate mineral salts include calcium chloride, calcium phosphate, sodium citrate, potassium hydroxide, potassium bicarbonate, ferrous sulfate, zinc sulfate, sodium chloride, copper sulfate, potassium iodide, sodium selenite, etc.. The mixture is then homogenized and cooled. Heat-labile vitamins and micronutrients may then be added to the mixture. The mixture is then standardized with deionized water to a final total solids

concentration of about 150 to about 160 and preferably about 155 grams per litre, which is equivalent to about 820 kcal per liter. The formula may be sterilized using a conventional ultrahigh temperature process, and then aseptically filled into appropriate packaging. Alternatively, the formula may be filled into glass jars and then retort sterilized.

It would be recognized by one skilled in the art that other known methods of manufacture and sterilization can be used for the preparation of the present infant formula. The present infant formula may also be produced as a concentrated liquid product requiring dilution with an equal volume of water prior to feeding to an infant. Furthermore such an infant formula may be dehydrated, such as in a spray dryer, to create a stable infant formula powder that offers advantages of stability and economy of transport, said powder requiring reconstitution with water prior to feeding to an infant.

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Examples

Example 1

A study was conducted to determine the total nucleotide concentration and the concentration of individual nucleotides in the breast milk collected from mothers of newborn, premature infants. The study was a prospective, non-randomized open study. Twenty seven mothers provided milk samples of volume greater than or equal to 20 ml.

The inclusion criteria for the study were as follows: outpatient, healthy women who were 18 to 40 years of age; parity less than 5; lactating; and between 4-18 days post partum on the scheduled day of collection. The study included mothers whose infants were born between 26-32 weeks gestational age, who were appropriate for gestational age and who weighed less than 1800 grams at birth.

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Samples of preterm human milk were analyzed for free nucleosides and free nucleosides after incubation with alkaline phosphatase. The increase in free nucleosides after the enzyme treatment represents the monomeric nucleotide

content on a molar basis. The concentrations in Table 1 below are expressed as equivalent monophosphates.

The results of this analysis are set forth in Table 1:

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Table 1

	Range (mg/L)	Mean (mg/L)
CMP	0.46 - 14.48	5.3
UMP	0.29 - 6.65	3.0
GMP	0.87 - 10.16	4.8
IMP	0.00 - 1.35	0.1
AMP	1.82 - 8.55	4.1
Total monomeric nucleotide	3.44 - 41.19	17.3

Example 2

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An example of the present infant formula is set forth below.

A typical 4,000 L production batch of ready-to-feed liquid infant formula mixture was manufactured in the following manner.

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A fat blend was prepared by combining 86.5 kg of Betapol (Loders Croklaan) 36.1 L of a high-oleic sunflower oil or safflower oil, 8.94 L of soybean oil, 24.7 kg of medium chain triglycerides, 1.36 kg of soy lecithin, 2.47 kg of an oil containing 38% of its fatty acids as arachidonic acid (ARASCO, Martek, Columbia, MD) and 1.65 kg of an oil containing 38% of its fatty acids as docosahexaenoic acid (DHASCO, Martek, Columbia, MD).

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The fat-soluble vitamins, vitamin A (vitamin A palmitate), beta-carotene, vitamin D (cholecalciferol), vitamin E (dl-alpha-tocopherol acetate) and vitamin K (phytonadione) were then dispersed in the fat blend.

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An amount of heated liquid skim milk or powdered skim milk sufficient to provide 44 kg of protein was added to warm deionized water in a compounding tank to which had been added 3620 g of sodium citrate and 1590 g potassium bicarbonate dissolved in hot water. The fat blend was metered into the compounding tank. The major dry ingredients, lactose, whey protein concentrate and maltodextrin, were added through a powder funnel eductor. The amount of added whey protein concentrate varied according to its protein content and was sufficient to provide 44 kg of protein. Taurine, 221 g was dissolved in hot water and added to the compounding tank. The following minerals were dissolved or dispersed separately in hot water and added to the compounding tank with intensive agitation: calcium phosphate, 2220 g; calcium chloride, 1710g; sodium chloride, 512g; potassium hydroxide, 643 g; ferrous sulfate, 153 g; potassium iodide, 72.5 g of a 1% triturate in lactose; 359 g of a 44.6% solution of zinc sulfate; 598 g of a 2.5% solution of copper sulfate and 7.22 g of a 0.3% sodium selenite triturate in potassium bicarbonate.

The mixture was heated to $96 \pm 2^\circ\text{C}$, held for 30 seconds and then cooled to $66 \pm 2^\circ\text{C}$. The mixture was then homogenized in a two-stage homogenizer, at 2500 psig in the first stage and 500 psig in the second stage. The homogenized mixture was then cooled to 5 - 10°C using a plate heat exchanger.

A sample of the mixture was analyzed for fat, pH and total solids. An appropriate amount of deionized water was added as required to achieve 80% of total dilution and the batch was mixed. A sample was taken for analysis of total solids and pH. The quantity of water required for final dilution was calculated. A portion of this dilution water was used to dilute the nucleotides and water-soluble vitamins before their addition to the mix. The following nucleotides were dissolved in warm water and added to the mix: cytidine 5' monophosphate, 31.7 g; adenosine 5' monophosphate, 27.9 g; uridine 5' monophosphate, disodium salt, 29.7 g; inosine 5' monophosphate, disodium salt, 5.43 g; and guanosine 5' monophosphate, disodium salt, 22.4 g.

Water-soluble vitamins were dissolved in warm water and were added to the mix as follows: Nicotinamide, 109 g; calcium pantothenate, 42.3 g; Thiamine hydrochloride, 8.98 g; Riboflavin, 3.55 g; Pyridoxine hydrochloride, 5.8 g; Folic acid, 2.51 g; D Biotin, 0.113 g; Cyanocobolamin, 7.24 g and myo-inositol, 104 g, were dissolved in water and added to the mix. Ascorbic acid, 1180 g, was dissolved in warm water and 535 g of potassium bicarbonate were added slowly to neutralize the ascorbic acid. The neutral ascorbate solution was then added to the mix. Citric acid, 106 g was dissolved in warm water and added to the mix. The mix was agitated for 30 minutes. The mix was standardized to total solids of about 123 g per litre.

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Twenty hours after the initial homogenization, the mix was homogenized again and sterilized through a Stork UHT (ultra high temperature) aseptic processing system and filled into 100 ml glass jars.

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The glass jars were then retort sterilized in a Barriquand rotary steriliser.

The ingredients of this formula were as follows:

	Ingredients	Units	/100 ml	/100 kcal
20	Protein	g	2.0	2.44
	Fat	g	4.4	5.37
	Carbohydrate	g	8.6	10.5
	Linoleic Acid	mg	550	671
	Energy	kcal	82	100
25	Vitamin A	mcg	90	110
	Vitamin D3	mcg	1.5	1.8
	Vitamin E	mg	1.2	1.5
	Vitamin K1	mcg	8	9.76
30	Vitamin B1	mcg	120	146
	Vitamin B2	mcg	200	244
	Vitamin B6	mcg	72	88
	Vitamin B12	mcg	0.3	0.37
	Niacin (NE)	mcg	820	1000
35	Folic Acid	mcg	48	59
	Pantothenic Acid	mcg	450	549
	Biotin	mcg	2.4	2.9
	Vitamin C	mg	11	13.4

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	Calcium	mg	80	97.6
	Phosphorus	mg	42.5	51.8
	Magnesium	mg	8	9.76
5	Iron	mg	0.8	0.98
	Zinc	mg	0.8	0.98
	Manganese	mcg	10	12.20
	Copper	mcg	82.5	101
	Iodide	mcg	10	12.2
10	Sodium	mg	35	42.7
	Potassium	mg	85	104
	Chloride	mg	60	73.2
	Choline	mg	15	18.3
15	Inositol	mg	4.5	5.49
	Taurine	mg	4.8	5.9
	CMP	mg	0.7	0.9
	UMP	mg	0.5	0.6
20	GMP	mg	0.4	0.5
	IMP	mg	0.1	0.12
	AMP	mg	0.6	0.7

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Example 3

The following infant formula according to the present invention was prepared according to the process described in Example 2.

30	Ingredients	Units	/100 ml	/100 kcal
	Protein	g	2.20	2.70
	Fat	g	4.23	5.20
	Carbohydrate	g	8.62	10.6
	Linoleic Acid	mg	550	677
35	Energy	kcal	81.3	100
	Vitamin A	mcg	224	275
	Vitamin D3	mcg	3.48	4.28
	Vitamin E	mg	4.09	5.03
40	Vitamin K1	mcg	6.1	7.5
	Vitamin B1	mcg	142	175
	Vitamin B2	mcg	203	250
	Vitamin B6	mcg	122	150
	Vitamin B12	mcg	0.33	0.41
45	Niacin (NE)	mcg	2353	2870

	Folic Acid	mcg	40.6	50.0
	Pantothenic Acid	mcg	1016	1250
	Biotin	mcg	3.25	4.00
5	Vitamin C	mg	14.6	18.0
	Calcium	mg	102.	125
	Phosphorus	mg	61	75.0
	Magnesium	mg	8.1	10.0
	Iron	mg	0.81	1.00
10	Zinc	mg	0.81	1.00
	Manganese	mcg	4.1	5.0
	Copper	mcg	91	112
	Iodide	mcg	24	30.0
	Sodium	mg	40.6	50.0
15	Potassium	mg	81	100
	Chloride	mg	73.2	90.0
	Selenium	mcg	1.71	2.10
	Choline	mg	13.0	16.0
	Inositol	mg	30.08	37.00
20	Taurine	mg	4.80	5.90
	CMP	mg	0.7	0.9
	UMP	mg	0.5	0.6
	GMP	mg	0.4	0.5
25	IMP	mg	0.1	0.12
	AMP	mg	0.6	0.7

30 The present invention may be embodied in other specific forms without
 departing from the spirit and essential attributes thereof and accordingly, reference
 should be made to the appended claims, rather than to the foregoing specification,
 as indicating the scope of the invention.